EFFECT OF VACUUM FREEZE-DRYING ON QUALITY OF LEMON BALM LEAVES *(Melissa officinalis,* L.*)*

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Abstract

This research focuses on the analysis of the chemical component of essential oil of lemon balm. The essential oil of leaves was obtained by extraction. The chemical components of the essential oil of lemon balm were analyzed by capillary GC and GC/MS and 8 substances were identified.

The quality of the final product depends mainly on the operation of freeze dryer (FD). It was found that essential oil content was more influenced by chamber pressure (high and low). The higher chamber pressure causes a longer drying period but results better quality volatile compounds of lemon balm. Comparative determinations of the essential oil in fresh and dried material at high pressure showed slightly higher content of the oil in the fresh one.

The drying data were fitted to thin-layer mathematical model (third-degree polynomial). The performance of this model was investigated by comparing the determination of coefficient (R^2) and root mean square error (RMSE) between the observed and predicted moisture ratios. The effect of freeze drying method on peltate glandular hairs structure was observed by electro-microscopy.

Key words: lemon balm, pressure, drying kinetics, essential oil, morphology

INTRODUCTION

Lemon balm (*Melissa officinalis,* L.) a member of Lamiaceae family is a perennial herb native to southern climates of Europe and North America (Sharafzadeh et al., 2011).

The leaves of *Melissa officinalis* contain relatively low essential oil, but the production cost and price of the oil are very high (Sari and Ceylan, 2002). The volatile oil content depending on provenances, nutrient, light intensity, variety, plant part age, harvesting stages, harvesting hours, oil isolation method and the drying method (Moradkhani et al., 2010, Cuervo-Andrade, 2011). Conventional drying brings undesirable changes in medicinal herbs. It is caused by several modifications in the material (chemical, physical, biological) as a consequence of the removal of moisture and increase of temperature during the drying procedure. Air-drying (temperature of 25-32°C for 10 days) caused a great reduction of antioxidant capacity of Melissa, reported by Capecka et al. (2005).

The development of new drying methods for medicinal herbs that allow reaching the required quality parameters. For example, the freeze drying can be used to avoid damage caused by heat, producing a product with superior physical and chemical qualities, it is considered a costly and time consuming process (Yousif et al., 2000).

The chemical constituents of volatile oil have been extensively studied. More than forty compounds were recognized in Melissa oil (Patora et al., 2003). Meftahizade et al. (2010) reported, that the main component of the essential oil in lemon balm leaves are citral (geranial and neral), citronellal, geraniol, beta-pinene, alpha-pinene, beta-caryophyllene, comprising 96% of the oil ingredients. The major compounds were citronellal and citral, accompanied by beta-caryophyllene, germacrene D, ocimene and citronellol, reported by Nykanen and Nykanen (1986).

There are many previous studies conducted on drying of various products. However, studies on drying characteristics of the lemon balm leaves are scarce in the literature. Moreover none of them reports about freeze drying kinetics and the effects of this drying technique in terms of chemical components of essential oil.

The aim of this study was to evaluate the qualitative and quantitative composition of volatile oil of the lemon balm. The effect of the pressure (high: FD-HP and low: FD-LP) of the freeze-drying procedure was also investigated.

MATERIAL AND METHOD

Raw material

The lemon balm was cultivated locally in Nyíregyháza and the plants were harvested just before flowering in June 2011. Fresh leaves were separated from the stem and only leaves were used for the drying and extraction of the oil. Essential oil was separately extracted from fresh leaves, freeze dried leaves at high pressure and freeze dried leaves at low pressure with three replications. The oil was identified by the staff at the Agrarian and Molecular Research Institute College of Nyíregyháza. The total amount of essential oil recovery was calculated in mg/100g based on the dry matter. The moisture content of fresh lemon balm was 5.09 kg water/kg dry matter (83.58% moisture content wet basis).

Determination of moisture content

To determine the moisture content of *Melissa officinalis* before and after freeze drying, the oven method (LP-306, LABOR-MIM, Hungary) was used. In this method the sample is placed inside and oven at 105°C for 24 h and the loss of mass is registered in order to determine the moisture content of the lemon balm. The test was carried out for triplicate.

Gas chromatography of the essential oil

Determinations of the volatile oil contents were done by chemical extraction (Moradkhani et al., 2010). About 50 g of fresh and ~9,5 g of dried plant leaves were subjected separately to extraction. The extraction procedure consisted of adding chloroform/hexane solvents (1:1, 600 ml) to the lemon balm samples, then mixing, blending and the ultrasonic homogenization of the sample (1 h, 40°C), followed by filtration and the release of solvent by rotating vacuum evaporation. The solvent was diluted with chloroform/hexane (1:1.5 ml) to allow for the release of chlorophyll with Al_2O_3. The remaining steps involved spraying the mixture with nitrogen gas, diluting the solvent with hexane (1 ml). A total of 1 µl of the extract was injected into the GC.

The oil was analyzed by Gas Chromatography (GC) and Gas Chromatography-mass Spectrometry (GC/MS). GC analysis was carried out on a Thermo Scientific Trace GC Ultra TG-5SILMS capillary column (30m×0.25mm, 0.25µm film thickness). The chromatographic conditions were as follows: The oven temperature increased from 40 (1 min) to 220°C (1 min) at a rate of 15°C/min, analysis time 14 min. The injector and detector temperature was 250°C. Helium used as the carrier gas was adjusted to linear velocity of 1.5 ml/min. The samples were injected using CT splitless method. Analytical standards of the flavour principles were obtained from Sigma-Aldrich. Quantitative data was obtained from electronic integration of peak areas without the use of correction factors.

GC/MS analysis was performed on a Thermo Scientific Trace GC Ultra-MS ITQ 1100 operating at 70eV ionization energy. It was equipped with a TG-5SILMS capillary column (30m×0.25mm, 0.25µm film thickness) with *Helium* as the carrier gas and a parameters of constant splitless injection: Temperature – 250°C, split flow – 10 ml/min, splitless time – 1 min. The temperature of MS transfer line: 270°C. The components of the oils were identified by both retention times and MS spectra. The result was an average of three determinations.

Drying procedure

The lemon balm leaves were lyophilized by freeze drying (FT33, Armfield, England). The product was dried for a period of 14 h at 200-300 Pa (FD-HP) and for 12 h at 50-80 Pa (FD-LP) with the heating plate kept at 18°C. The condenser temperature was kept at –50 to –55°C. A special digital weighing apparatus (EMALOG, Hungary, accuracy of 5000 ±0.1 g) measures the mass loss of the product during the freeze drying process. During each drying experiment, the weight of the samples on the tray was measured. The tests were repeated three times. The final moisture content of dried lemon balm leaves: 0,15 kg water/kg dry matter (10.92% moisture content wet basis).

Mathematical modelling of drying curve

The third-degree polynomial equation (1) is the most convenient mathematical model of freeze drying kinetics and has been used in thin layer drying studies.

$$MR=at^3+bt^2+ct+1 \tag{1}$$

The values of parameters *a, b, c* of the third-degree polynomial depend on the characteristics of the material, including variety, freezing rate, ripeness, and tendency to lose water (Antal et al., 2011).

The moisture ratio (MR) of lemon balm during drying experiments was calculated using the following equations (2):

$$MR = \frac{M - M_e}{M_0 - M_e} \tag{2}$$

where, MR is dimensionless moisture ratio, M is moisture content dry basis (kg water/kg dry matter), M_e is equilibrium moisture content dry basis (kg/kg db), M_0 is initial moisture content dry basis (kg/kg db).

The sample moisture content M was calculated on a dry basis (db) according to equation (3):

$$M = \frac{W_t - W_k}{W_k} \tag{3}$$

where, M is moisture content dry basis (kg water/kg dry matter), W_t is sample weight at a specific time (kg), W_k is sample dry weight (kg).

The correlation coefficient (R^2) was primary criterion for selecting the best equation to describe the drying curve equation. In addition to R^2, the root mean square error analysis (RMSE) was used to determine the goodness of the fit (4, 5).

$$R^2 = \frac{\text{Residual sum of squares}}{\text{Corrected total sum of squares}} \tag{4}$$

$$RMSE = \sqrt{\frac{1}{N} \cdot \sum_{i=1}^{N} (MR_{\exp_i} - MR_{pre_i})^2} \tag{5}$$

where, $MR_{exp,i}$ is the experimental moisture ratio at observation, $MR_{pre,i}$ is the predicted moisture ratio at this observation, N is number of observations.

The lower RMSE values and the higher R^2 values indicate the high fit of the model.

Microstructure imaging

Fresh and freeze dried leaves of lemon balm were examined by BRESSER BIOLUX AL type electro-microscope. The leaves were broken into smaller pieces. We took photos of (4× and 10×) magnification with the program named MicrOcular. Through the camera attached to the microscope we transmitted the photos to the computer.

Statistical analysis

Tukey's test was used to determine significant differences ($p<0,05$) between the three types of lemon balm (fresh, FD at high pressure, FD at low pressure). The statistics package chosen was PASW Statistics version 18.0 (SPSS Inc., USA).
The software package Microsoft Office Excel 2007 was used in the numerical calculations.

RESULTS AND DISCUSSIONS

Drying kinetics

The dimensionless moisture content (MR) change during freeze drying is presented in Figure 1. In this figure the experimental data and the mathematical modelling of the drying kinetics experimental data for lemon balm samples are also shown.

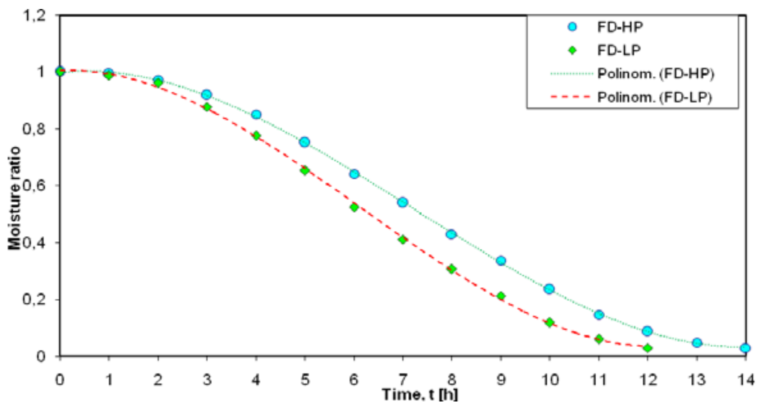

Fig. 1 Moisture ratios versus time at various chamber pressures

This figure shows higher drying rate when the sample is dried at low chamber pressure (FD-LP). The final moisture content for *Melissa officinalis* was found to be about 0,15 kg water/kg dry matter (db) after 12 and 14 hours of drying time for FD-LP and FD-HP samples, respectively.

The final dimensionless moisture content of the products for FD-LP and FD-HP samples were 0.029. Thus, FD-HP samples required 2 hours longer drying time to achieve the same final moisture content compared to FD-LP Melissa samples.

The Table 1 shows the drying model coefficients and the comparison criteria used to assess quality of fit. The statistical values, R^2 and RMSE, are also shown in Table 1. The R^2 and RMSE values changed between 0.9994 and 0.9998, 5.24×10^{-3} and 1.022×10^{-2}, respectively. So, the model could be used to describe the drying of lemon balm leaves.

The drying kinetics results showed that there is a good agreement between the experimental data and thin-layer modelling equations. As shown in Table 1, the highest value of coefficient of determination ($R^2>0.999$) and the lowest value of the root mean square error less than 1.022×10^{-2} predicted by third-degree polynomial's model for Melissa samples.

Table 1

Effect of drying conditions on model parameters

Drying method	Model name	Model parameters		
		a	b	c
FD-HP[1]	Polynomial	0,0008	-0,0177	0,0187
FD-LP[2]		0,0012	-0,0213	0,0089

[1] Freeze dried at high pressure
[2] Freeze dried at low pressure

This model can be used satisfactorily to predict the experimental values of the moisture ratio values.

Our previous results indicate that in the case of the freeze drying the polynomial model gives the most accurate fit (Antal et al., 2011a).

Identification of essential oil components

The chemical composition of lemon balm oil was characterized by Gas Chromatography (GC) and GC/MS analysis. In Figure 2 the chromatogram of fresh lemon balm leaves essential oil are presented from mass spectral data. The principal components of volatile oil of the leaves: citral, beta-caryophyllene, beta-cis-ocimene, alpha-calacorene.

Table 2 presents the results of GC in the fresh and lyophilized lemon balm oil. The major volatiles found were citral, citronellal, geraniol and limonene. The main composition of the fresh leaf essential oil agreed with the data of the previous authors (Schnitzler et al., 2008).

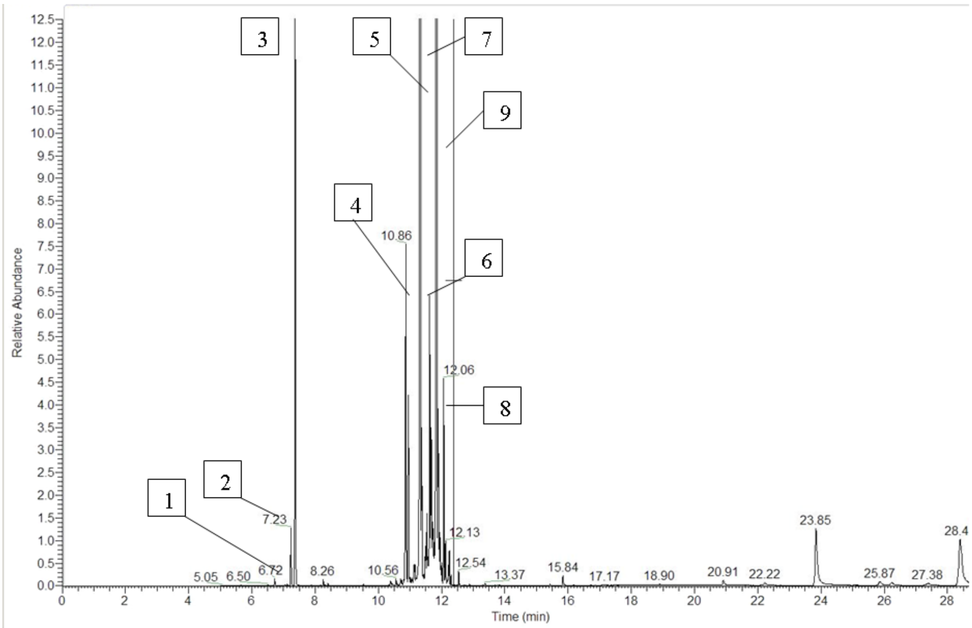

Fig. 2 Gas-Chromatogram of volatiles extract from fresh ‘Melissa officinalis’
The peaks correspond to identified compounds: (1) β-pinene, (2) limonene, (3) β-cis-ocimene, (4) α-cubebene, (5) β-caryophyllene, (6) α- caryophyllene, (7) citral, (8) δ-cadinene, (9) α-calacorene

The citral, citronellal and geraniol as major chemical compositions of the essential oil of the lemon balm have been previously reported (Sari and Ceylan, 2002). Meftahizade et al. (2010) reported that the main constituents of the essential oil are citral, citronellal, geraniol, beta-pinene, alpha-pinene, beta-caryophyllene, comprising 96% of the oil ingredients.

Table 2

Volatile compounds of lemon balm leaves

Components [mg/100g db]	Concentration		
	Fresh	FD-HP[1]	FD-LP[2]
Citral	123,65[a]	108,34[b]	81,59[c]
Citronellal	77,33[a]	66,56[b]	54,90[c]
Geraniol	29,12[a]	28,02[a]	16,88[b]
Limonene	23,79[a]	17,89[b]	12,13[c]
β-citronellol	14,87[a]	10,32[b]	9,64[b]
β-pinene	11,48[a]	10,62[ab]	6,91[b]
Linalool	10,23[a]	8,74[ab]	6,85[b]
Terpineol	2,53[a]	2,27[a]	2,09[a]
Total [%]	0,293[a]	0,252[b]	0,191[c]

[1] Freeze dried at high pressure
[2] Freeze dried at low pressure
[abcd] Different letters in the same row indicate statistical differences at the 0,05 level according to the Tukey’s test.

Also Carnat et al. (1998) reported the chemical composition of essential oil of lemon balm, and found that major components are citral representing 48,2% of the essential oil, followed by citronellal with 39,7% and caryophyllene with 2,37%. The composition of the leaf essential oil agreed with the data of the previous authors.

Our study revealed that FD-HP samples retained most of the compounds significantly better than FD-LP samples. This means that the concentrations of essential oils in the FD-processed herb decreased with a reduction of pressure in the drying chamber of the freeze dryer.

Morphology of glandular hairs (leaves)

Microscope pictures were taken of Melissa leaves both before and after freeze drying. This was to investigate whether or not there are any structural changes in the oil reservoirs during freeze drying.

The essential oil is located in oil reservoirs, such as peltate glands and glandular hairs shed. The vegetative organs of *Melissa officinalis* (leaves) are containing glandular needle-shaped trichomes. This statement was in agreement with literature (Cuervo-Andrade, 2011). Figure 3. shows the glandular hair on fresh leaf of lemon balm (10× magnification). The essential oil is found on the surface of the leaves in peltate glandular trichomes.

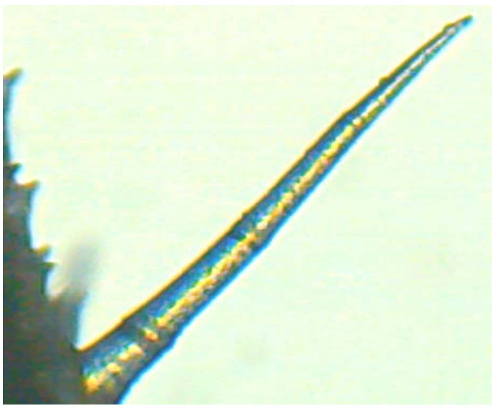

Fig. 3 Microphotograph of raw glandular trichome

When freeze dried at low pressure (50-80 Pa) occur a few of the glandular trichomes appeared slightly split open, suggesting oil loss, while most of them appeared to be damaged (Fig. 4.). The reduction in essential oil yield is associated with the observed loss of glandular contents, is attributed to the evaporation of volatile components. This is in agreement with above chapter, the highest losses in volatiles occurred in the freeze dried (at low pressure) samples.

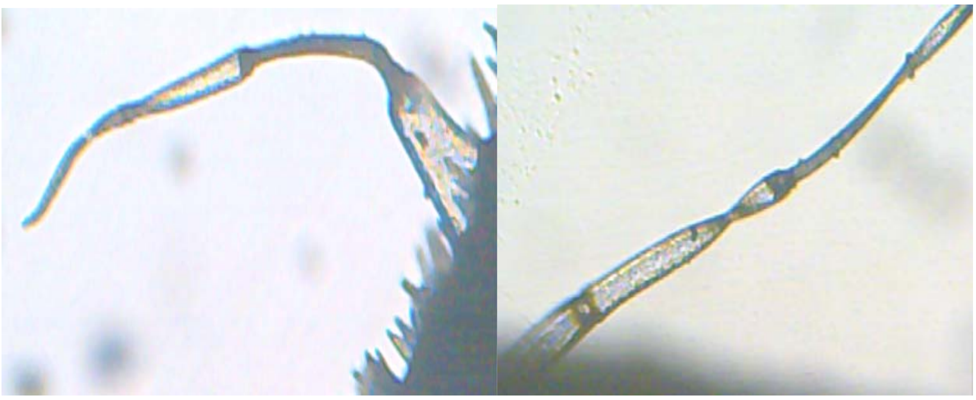

Fig. 4 Microphotograph of glandular hairs subjected to freeze drying at low pressure

The glandular trichomes remained relatively plump and the change of shape is minimum for the freeze drying at high pressure (200-300 Pa). Thus, the trichome structure in these samples is similar to that in the fresh samples. The Figure 5. demonstrates the effect of high pressure on glandular hairs by microscopic view (10× magnification).

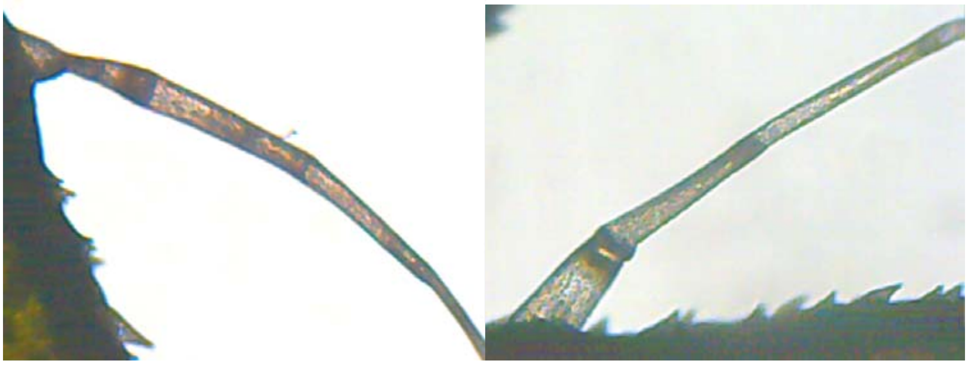

Fig. 5 Microphotograph of glandular hairs subjected to freeze drying at high pressure

CONCLUSIONS

The volatiles composition of freeze dried lemon balm under different conditions of pressure has been studied. Essential components for the *Melissa officinalis* were detected in fresh samples as well as in dried ones. The essential oil of the fresh material was higher than of the dried one. The quality of lemon balm extract obtained from FD-HP (freeze dried at high pressure) samples was found to be superior, as compared to that of FD-LP (freeze dried at low pressure) samples. From the drying kinetics result, the FD-LP lemon balm leaves had shorter drying time than the FD-HP samples. A decrease in drying chamber pressure significantly decreased the freeze-drying time of the lemon balm leaves but considerably increased the release of volatile compounds.

The third-degree polynomial equation has shown a very well fit to experimental data. According to the results, the polynomial equation could adequately describe freeze drying characteristics of lemon balm leaves.

The electro-microscope pictures before and after freeze drying at high pressure showed that there were little changes (moderate) in the glandular trichomes. After freeze drying at low pressure, hairs exhibited significant changes in their structures such as split open and volatile oils evaporate to the air. This confirm the statement of our, that the amount released is dependent on the pressure of freeze drier.

We recommend the drying of lemon balm leaves by freeze drying and the pressure in the drying chamber should not be too low.

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